

Directive

9181.1

2-26-01

TESTING FOR STARLINK™ CORN - LATERAL FLOW TEST STRIP METHOD

1. PURPOSE

This directive establishes official procedures for testing corn (not processed corn products), using the lateral flow strip method, for the presence of StarLink™ corn and certifying the official results. This service is provided as official criteria under the authority of the United States Grain Standards Act (USGSA), as amended.

All official testing is performed as prescribed in this directive by authorized employees of the Federal Grain Inspection Service (FGIS) or licensed delegated/designated agency personnel. Individuals wanting official services should contact the nearest FGIS field office or delegated/designated agency. See section 12 for details.

2. REPLACEMENT HIGHLIGHTS

This directive is revised to modify the StarLink™ certification statements, establish a 2400-kernel sample as the standard sample size for testing sublots on export ships and barges, and to include testing procedures for performing StarLink™ testing on the basis of 800-kernel portions. Additionally, FGIS is redefining the meaning of a "subsample" for qualitative StarLink™ testing using Strategic Diagnostics Incorporated (SDI) and Envirologix test kits. This directive supersedes FGIS Program Directive 9181.1, dated 1/26/01, and FGIS Policy Bulletin Board Notice Number 191, dated 2-2-01.

3. BACKGROUND

StarLink™ corn, developed by Aventis CropScience, was developed through modern biotechnology by introducing genetic material from a bacterium, *Bacillus thuringiensis* (*Bt*). As a result, StarLink™ corn produces a protein, Cry9C, which has insecticidal properties effective in controlling the European corn borer. The Environmental Protection Agency (EPA) approved StarLink™ in 1998 for use as animal feed only.

FGIS has verified the performance of two lateral flow test kits, the Trait✓ Bt9 Lateral Flow Test Kit, manufactured by SDI, and the Cry9C QuickStix™ Test Kit manufactured by EnviroLogix Incorporated, to detect the presence of the Cry9C protein in corn. *(The mention of firm names or trade products does not imply that the U. S. Department of Agriculture endorses or recommends them over other firms or similar products not mentioned.)*

The test protocol does not determine the exact percent of StarLink™ kernels present in the sample. It determines the probability that a sample contains or does not contain the Cry9C protein at a specified concentration based on the number and size of samples tested. See section 4 for further information.

4. TESTING CONFIDENCE

Based on sampling theory, lot size is an insignificant influence on sample size when the lot size is very large relative to the sample sizes. This is generally the case in grain inspection. The total sample size is dependent on the probability of accepting a specified concentration. The limitations of the analytical method determine how many sub-samples have to be tested. For example, if an applicant wants a sample of 2400 kernels tested but the analytical method can only reliably detect one kernel in a sample of 800 kernels, then the 2400-kernel sample must be tested as three sub-samples of 800 kernels.

Official sampling procedures have been developed as practical procedures to obtain samples that approximate random samples. Increasing the sample size can increase the confidence of detection at lower levels; however, the results of a larger sample size may only be reliably achieved by testing the sample as multiple sub-samples.

The following table illustrates the confidence levels achieved for the approved test kits based on the total number of kernels tested. The table provides information at five confidence levels regarding maximum percent StarLink™ levels that would be expected in the sample if all test samples provide negative results.

Total Kernels Tested	Percent StarLink™ at Five Different Confidence Levels (%)				
	Note: All Sub-Samples Must be Negative				
	50	75	90	95	99
400	0.17	0.35	0.58	0.75	1.2
800	0.087	0.18	0.29	0.38	0.58
1200	0.058	0.12	0.20	0.25	0.39
1600	0.045	0.085	0.15	0.19	0.29
2000	0.035	0.070	0.12	0.15	0.25
2400	0.029	0.058	0.10	0.13	0.20
2800	0.025	0.050	0.085	0.11	0.18
3200	0.022	0.044	0.075	0.10	0.15

If 400 kernels are analyzed and the result is negative for the Cry9C protein, a 99 percent probability exists that a lot does not contain more than 1.2 percent StarLink™ corn. Likewise, if 800 kernels are analyzed and results are negative for the Cry9C protein, a 99 percent probability exists that a lot does not contain more than 0.58 percent StarLink™ corn.

Since different users of corn have different needs, FGIS will analyze the total number of kernels as requested by the applicant for service. See section 12 for additional information regarding the biotechnology sample planner.

5. WORK AREA REQUIREMENTS

The work area requirements covered under this section apply to FGIS-occupied space only.

Testing may be performed in the laboratory space provided for mycotoxin analysis or in other suitable locations (e.g., tabletop in inspection lab) as determined by the FGIS manager.

6. TYPES OF SERVICES

Three types of testing services are available as follows:

- a. Submitted Sample Service. Analysis based on a sample submitted by the applicant for service.
- b. Official Sample-Lot Service. Analysis based on an official sample obtained and analyzed by someone authorized or licensed by FGIS.
- c. Warehouse Sample-Lot Inspection Service. Analysis based on an official sample obtained by a licensed warehouse sampler and analyzed by someone authorized or licensed by FGIS.

7. SAMPLE PREPARATION

- a. Precautions.

Inspection personnel must employ additional care to protect against inadvertent commingling and to ensure sample integrity. A single kernel commingled with the official sample could affect test results. Ensure sample collection devices (e.g., sampling mat, collection buckets) and laboratory equipment (sample bags, containers, and dividers) are free from residual grain.

- b. Sample Size.

Obtain samples according to the procedures in the Grain Inspection Handbook, Book I, "Grain Sampling." See section 12 for details. Use an approved divider to obtain the required analytical portion(s). Ensure the original sample size is sufficient to accommodate the requested analysis and provide for a file sample. General class scales are acceptable for weighing sample portions.

FGIS has adopted 2400 kernels as the standard sample size for StarLink™ testing on sublots for export ships and barges. Upon request of the applicant, official personnel may perform the testing on the basis of an alternate sample size (e.g., 1200 kernels). For all other types of samples (e.g., submitted samples, trucklots) the applicant must state the sample size (e.g., 400 kernels, 800 kernels).

Use the following table to determine the number of grams per test portion, or calculate the analytical portion (in grams) by weighing 100 kernels then multiplying the weight (in grams) by the appropriate sample size. For example; to determine the number of grams in an 800-kernel sample size, weigh 100 kernels (in grams), then multiply the weight by 8.

<u>Test Portion (Kernels)</u>	<u>Test Portion (Grams)</u>
100 kernels	29 (\pm 5 grams)
200 kernels	58 (\pm 5 grams)
300 kernels	86 (\pm 5 grams)
400 kernels	115 (\pm 5 grams)
500 kernels	143 (\pm 5 grams)
600 kernels	172 (\pm 5 grams)
700 kernels	200 (\pm 5 grams)
800 kernels	229 (\pm 5 grams)

c. Defining Subsample Size.

A "subsample" is defined as a sample portion of up to 800 kernels (approximately 229 grams) in size that is used to perform StarLink™ testing. Simplified, each lateral flow test strip used for a test is considered as a subsample. For example, at a detection level of 1 in 2400 kernels, use 3 subsamples of 800 kernels (3 test strips capable of detecting at the 0.125 sensitivity level) or 6 subsamples of 400 kernels.

d. Basis of Testing (Analytical Portion).

Base the analysis on the sample as a whole (before the removal of broken corn and foreign material), unless otherwise specified by the applicant for service.

8. TEST KITS

FGIS has verified the performance of lateral flow test kits manufactured by SDI, and EnviroLogix Incorporated. The Cry9C QuickStix™ Test Kit manufactured by EnviroLogix can detect the presence of the Cry9C protein in corn at a level of 0.125 percent (1 kernel in 800).

The Trait✓ Bt9 Lateral Flow Test Kit, manufactured by SDI, has two separate test kits available for testing at the 0.25 percent (1 kernel in 400) and the 0.125 percent (1 kernel in 800) detection levels. SDI test kits identified with part number 7000034 are restricted to testing at the 1 in 400-kernel level. Test kits identified with part number 7000003 are approved for testing at the 1 in 800-kernel level.

NOTE: SDI Test Kits (part number 7000003) do not include buffer solution.

9. TRAIT✓Bt9 TESTING PROCEDURES

a. Processing the Sample - Part number 7000034 (1 kernel in 400)

- (1) Place each sub-sample 115 grams (± 5 grams), (approximately 400 kernels) in a **clean, dry "Mason" jar or similar container** used for blending purposes. (**Container must hold a minimum of 16 fluid ounces.**)
- (2) Attach the container and **clean, dry cutting blades** to the mixing device (food processor or blender). Place a plastic shield over the container (for protection) in case the container breaks during sample processing.
- (3) Grind the sub-sample on high speed until all whole kernels are broken (approximately 30 seconds).

Note: An alternative method to grind the sub-sample is permitted provided the particle size is comparable to the blender procedure and processes are in place to prevent cross-sample contamination.

- (4) Remove the sub-sample container from the mixing device and add 143 to 145 milliliters (ml) of water.

NOTE: If the analytical portion size was adjusted to approximate 400 kernels, adjust the water volume using the formula:

Sample weight (grams) X 1.25 = Water volume (ml)

- (5) Place a lid on the container and shake to thoroughly wet all corn particles (approximately 10 - 20 seconds). Let the slurry settle for 15 - 30 seconds. The sub-sample will have a thick consistency but should contain some free liquid.
- (6) Transfer 0.5 ml of the free liquid from the container and place in a 1.5-ml sample tube using a transfer pipette provided with the test kit.

Note: Some corn material will be transferred with the free liquid in this step.

- (7) Add 5 drops of the Trait✓ Sample Buffer into the sample tube.

Caution: Failure to add 5 drops of buffer to the tube may cause false positive results.

- (8) Close the tube and shake for approximately 10 seconds.

- (9) Place 1 Trait✓ Bt9 Test Strip into the sample tube. **Allow the strip to remain in the test tube for a full 10 minutes.** If a control line (top of the strip) does not develop the test is invalid. Reading the strip prior to 10 minutes could result in false negative results.

Caution: The top of the liquid in the sample tube should not be higher than the top of the arrows on the test strip when it is in the sample tubes.

b. Alternate Test Levels.- Part number 7000034 (1 kernel in 400)

Testing can be performed at different levels (e.g., 1 in 300 kernels, 1 in 200 kernels) by reducing the amount of sample and water volume. Use the following table to determine the appropriate sample size and water volume for alternate test levels.

Test Level	Sample Size (Kernels)	Sample Size (Grams)	Water Volume
1 in 300	300 kernels	86 (\pm 5 grams)	108 - 110 ml
1 in 200	200 kernels	58 (\pm 5 grams)	72 - 74 ml
1 in 100	100 kernels	29 (\pm 5 grams)	36 - 38ml

c. Processing the Sample - Part number 7000003 (1 kernel in 800)

- (1) Place each sub-sample 229 grams (\pm 5 grams), (approximately 800 kernels) in a **clean, dry "Mason" jar or similar container** used for blending purposes. (**Container must hold a minimum of 32 fluid ounces.**)
- (2) Attach the container and **clean, dry cutting blades** to the mixing device (food processor or blender). Place a plastic shield over the container (for protection) in case the container breaks during sample processing.
- (3) Grind the sub-sample on high speed until all whole kernels are broken (approximately 30 seconds).

Note: An alternative method to grind the sub-sample is permitted provided the particle size is comparable to the blender procedure and processes are in place to prevent cross-sample contamination.

- (4) Remove the sub-sample container from the mixing device and add 286 to 288 milliliters (ml) of water.

NOTE: If the analytical portion size was adjusted to approximate 800 kernels, adjust the water volume using the formula:

Sample weight (grams) X 1.25 = Water volume (ml)

- (5) Place a lid on the container and shake to thoroughly wet all corn particles (approximately 10 - 20 seconds). Let the slurry settle for 15 - 30 seconds. The sub-sample will have a thick consistency but should contain some free liquid.
- (6) Transfer 0.5 ml of the free liquid from the container and place in a 1.5-ml sample tube using a transfer pipette provided with the test kit.

Note: Some corn material will be transferred with the free liquid in this step.

- (7) Place 1 Trait✓ Bt9 Test Strip into the sample tube. **Allow the strip to remain in the test tube for a full 10 minutes.** If a control line (top of the strip) does not develop the test is invalid. Reading the strip prior to 10 minutes could result in false negative results.

Caution: The top of the liquid in the sample tube should not be higher than the top of the arrows on the test strip when it is in the sample tubes.

d. Alternate Test Levels.- Part number 7000003 (1 kernel in 800)

Testing can be performed at different levels (e.g., 1 in 400 kernels, 1 in 600 kernels) by reducing the amount of sample and water volume. Use the following table to determine the appropriate sample size and water volume for alternate test levels.

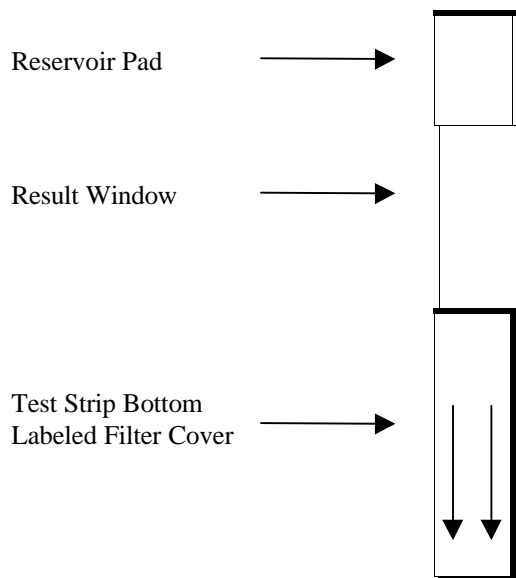
Test Level	Sample Size (Kernels)	Sample Size (Grams)	Water Volume
1 in 700	700 kernels	200 (\pm 5 grams)	250 - 252 ml
1 in 600	600 kernels	172 (\pm 5 grams)	215 - 217 ml
1 in 500	500 kernels	143 (\pm 5 grams)	179 - 181 ml
1 in 400	400 kernels	115 (\pm 5 grams)	143 - 145 ml
1 in 300	300 kernels	86 (\pm 5 grams)	108 - 110 ml
1 in 200	200 kernels	58 (\pm 5 grams)	72 - 74 ml
1 in 100	100 kernels	29 (\pm 5 grams)	36 - 38 ml

e. Interpreting the Lateral Flow Test Strip.- Part numbers 7000003 and 7000034

Check the result window frequently after inserting the strip. At least one line, the Control Line, should always develop approximately 1 centimeter down from the Reservoir Pad. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a positive result.

NOTE: Development of the Control Line serves only to indicate that the device has functioned properly. Discard any test strip that does not develop a Control Line and re-test the sample using another strip.

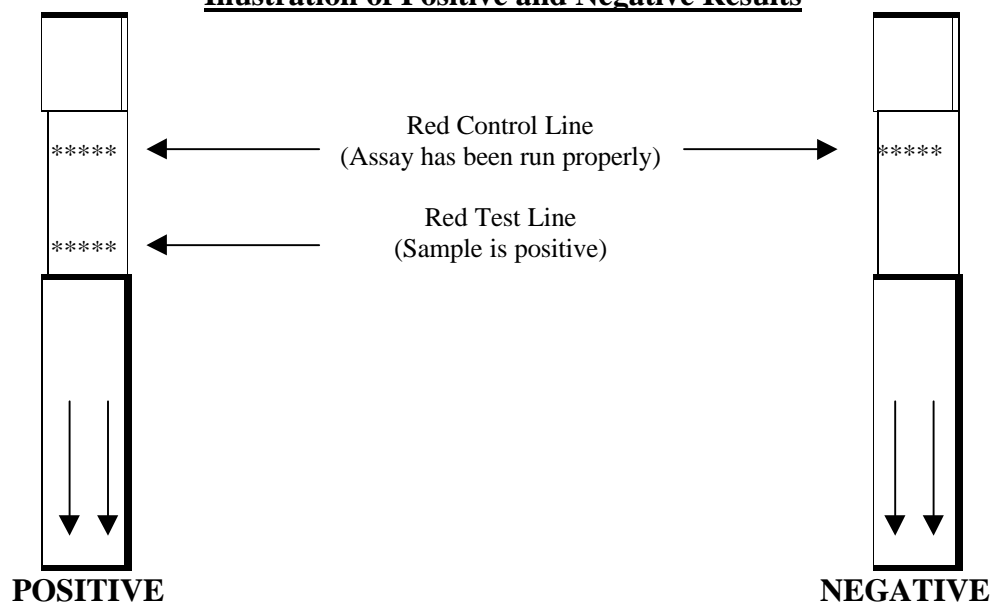
Illustration of Test Strip



As illustrated in the example below, the appearance of **one line** (control) on the strip indicates that the sample is **negative** for StarLink™ corn.

The appearance of **two lines** on the strip indicates that the sample is **positive** for StarLink™ corn.

Illustration of Positive and Negative Results



NOTE: If the sample contains high levels of StarLink™ corn, the control line may be faint and the test line very strong in color.

f. Reporting and Certifying Test Results.

See section 11 for detailed certification procedures.

g. Equipment Cleaning and Waste Disposal.

- (1) Clean mixing containers and cutting blades with soapy water, rinse with clean water, and thoroughly dry before reusing. **Failure to do this could result in cross-contamination resulting in errors.**
- (2) Dispose solid material (e.g., test strips, sample tubes, ground corn) into a solid waste container for routine disposal. Liquid materials (excess sample solution) may be poured down the drain.

h. Equipment and Supplies.

- (1) Test Kit Materials
 - (a) Trait✓ Bt9 Lateral Flow Test Strips
 - (b) Trait✓ Sample Buffer (included with kit number 7000034 only)
 - (c) Sample Tubes (1.5 ml)
 - (d) Transfer Pipettes
- (2) Laboratory Equipment
 - (a) Blender/processor equipped with cutting blades, gasket, and mixing container
 - (b) Plastic blender shield
 - (c) Sample tube rack
 - (d) Graduated cylinder

i. Storage Conditions.

Store the Trait✓ Bt9 Test Kits at room temperature (60° to 85°F). Storage conditions higher than room temperature may adversely affect performance. Keep the test strips in the foil pouch with the indicating desiccant. Do not use the test strips or the buffer if the desiccant is not blue in color.

10. QUICKSTIX™ TESTING PROCEDURES

a. Processing the Sample.

- (1) Place each sub-sample 229 grams (± 5 grams), (approximately 800 kernels) in a **clean, dry "Mason" jar or similar container** used for blending purposes. (**Container must hold a minimum of 32 fluid ounces.**)
- (2) Attach the container and **clean, dry cutting blades** to the mixing device (food processor or blender). Place a plastic shield over the container (for protection) in case the container breaks during sample processing.
- (3) Grind the sub-sample on high speed until all whole kernels are broken (approximately 30 seconds).

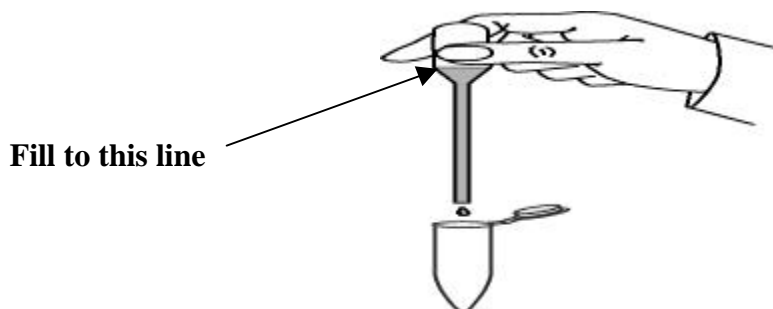
Note: An alternative method to grind the sub-sample is permitted provided the particle size is comparable to the blender procedure and processes are in place to prevent cross-sample contamination.

- (4) Remove the sub-sample container from the mixing device and add 366 to 368 milliliters (ml) of **distilled or deionized water**.

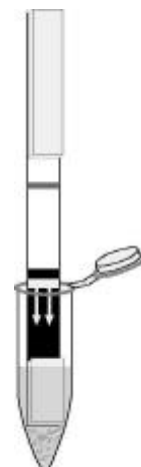
NOTE: If the analytical portion size was adjusted to approximate 800 kernels, adjust the water volume using the formula:

Sample weight (grams) X 1.6 = Water volume (ml)

- (5) Place a lid on the container and shake to thoroughly wet all corn particles (approximately 10 - 20 seconds). Let the slurry settle for 15 - 30 seconds. The sub-sample will have a thick consistency but should contain some free liquid.
- (6) Using the transfer pipette provided with the test kit, draw up enough liquid portion to fill the long narrow tip of the pipet up to the line at the top of the pipet bulb. Avoid pulling up particles of corn.



- (7) Dispense the extract into a reaction tube.
- (8) Place 1 QuickStix™ Test Strip into the sample tube. After inserting the strip into the reaction tube you will observe liquid travelling up the membrane strip toward the absorbent pad at the top of the strip. **Allow the strip to remain in the test tube for a full 10 minutes.** If a control line (top of the strip) does not develop the test is invalid. Reading the strip prior to 10 minutes could result in false negative results.



b. Alternate Test Levels.

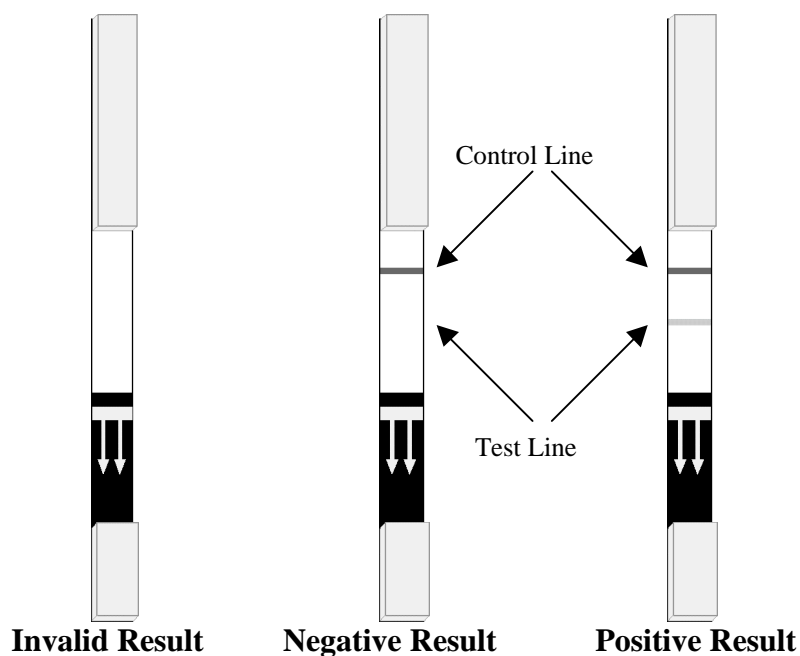
Testing can be performed at different levels (e.g., 1 in 400 kernels, 1 in 600 kernels) by reducing the amount of sample and water volume. Use the following table to determine the appropriate sample size and water volume for alternate test levels.

Test Level	Sample Size (Kernels)	Sample Size (Grams)	Water Volume
1 in 700	700 kernels	200 (\pm 5 grams)	320 - 322 ml
1 in 600	600 kernels	172 (\pm 5 grams)	275 - 277 ml
1 in 500	500 kernels	143 (\pm 5 grams)	229 - 231 ml
1 in 400	400 kernels	115 (\pm 5 grams)	183 - 185 ml
1 in 300	300 kernels	86 (\pm 5 grams)	138 - 140 ml
1 in 200	200 kernels	58 (\pm 5 grams)	92 - 94 ml
1 in 100	100 kernels	29 (\pm 5 grams)	46 - 48ml

c. Interpreting the Lateral Flow Test Strip.

At least one line, the Control Line, should always develop. A red line in this position indicates that the device is functioning properly. A red line appearing below the Control Line is the Test Line and indicates a positive result.

NOTE: Development of the Control Line serves only to indicate that the device has functioned properly. Discard any test strip that does not develop a Control Line and re-test the sample using another strip.



d. Reporting and Certifying Test Results.

See section 11 for detailed certification procedures.

e. Equipment Cleaning and Waste Disposal.

- (1) Clean mixing containers and cutting blades with soapy water, rinse with clean water, and thoroughly dry before reusing. **Failure to do this could result in cross-contamination resulting in errors.**
- (2) Dispose solid material (e.g., test strips, sample tubes, ground corn) into a solid waste container for routine disposal. Liquid materials (excess sample solution) may be poured down the drain.

f. Equipment and Supplies.

- (1) Test Kit Materials
 - (a) QuickStix™ Test Strips
 - (b) Sample Tubes
 - (c) Transfer Pipettes

(2) Laboratory Equipment/Supplies

- (a) Blender/processor equipped with cutting blades, gasket, and mixing container (minimum 32 ounce)
- (b) Plastic blender shield
- (c) Sample tube rack
- (d) Graduated cylinder (minimum size: 400 ml)
- (e) Distilled or deionized water

g. Storage Conditions.

Store the QuickStix™ Test Kits at controlled room temperature (64° to 77° F) or refrigerated (39° to 46° F) for longer shelf life. Protect the test kits from excessive humidity and extreme hot and cold temperatures when not in use. **Do not open the dessicated cannister until you are ready to use the test devices.**

11. CERTIFICATIONa. Standard Certification Statements.

Report the test results on the certificate with the appropriate statement:

(1) All Sub-Samples are Negative.

"Negative StarLink test result based on (total number) kernels ([number] sub-samples)."

Example: "Negative StarLink test result based on 2400 kernels (3 sub-samples)."

(2) Any Sub-Sample is Positive.

"Positive StarLink test result based on (total number) kernels ([number] sub-samples)."

Example: "Positive StarLink test result based on 800 kernels (1 sub-sample)."

b. Additional Statements.

(1) Composite Sample Testing on Export Lots

If an applicant requests StarLink testing on an export lot (ship, barge, or railcar) on the basis of a composite sample, use the following statement in addition to the standard certification statement.

"Results based on the analysis of a composite sample of the lot."

(2) Reporting Confidence Levels

If an applicant requests the testing confidence level reported on the certificate use the following statement.

"Test results determined at the (level) confidence level."

12. ADDITIONAL INFORMATION

The following information is available from the USDA web site:

a. Official Service Providers.

<http://www.usda.gov/gipsa/aboutus/servicemap/usmap.htm>

b. Biotechnology Sample Planner.

<http://www.usda.gov/gipsa/biotech/biotech.htm>

c. Official Grain Sampling Procedures.

<http://www.usda.gov/gipsa/reference-library/handbooks/grain-insp/grbook1/gihbk1.htm>).

Contact the Policies and Procedures Branch at 202-720-0252 if additional information is needed.

/s/ David Orr

David Orr, Director
Field Management Division